



[APPENDIX II]

300. A method of treating tissue to prevent or control air or fluid leaks comprising:

providing a composition to tissue, said composition including an albumin protein at about 20-60 wt/vol% and a crosslinking agent at about 50-800 mg/ml, said crosslinking agent having a polyoxyethylene chain portion and an activated leaving group which allows the crosslinking agent to react with said protein and having a molecular weight of about 1000-15,000; and

curing said composition on the tissue to bond said composition to the tissue and to provide a substantive cured matrix.

301. The method of claim 300 wherein said composition is cured to produce the matrix in less than about 10 minutes.

302. The method of claim 300 wherein said composition is cured to produce the matrix in less than about one minute.

303. The method of claim 300 wherein said composition is cured to produce the matrix in about ten seconds.

304. The method of claim 300 comprising providing the composition to the tissue using a syringe.

305. The method of claim 300 comprising providing the composition to the tissue using a dual syringe.

306. The method of claim 300 comprising providing the composition to the tissue using a spray apparatus.

307. The method of claim 300 wherein the matrix is resorbed.

308. The method of claim 307 wherein the matrix is resorbed in about four to sixty days.

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309. The method of claim 300 comprising curing the composition such that the peel strength of the matrix is about 0.08 lb/in or more.

310. The method of claim 300 comprising curing said composition to provide a cured matrix that has a burst pressure greater than about 10 mmHg.

311. The method of claim 309 wherein the matrix has a burst pressure of about 34 mmHg or greater.

312. The method of claim 311 wherein the matrix has a burst pressure of about 90 mmHg or greater.

313. The method of claim 312 wherein the matrix has a burst pressure of about 130 mmHg or greater.

314. The method of claim 300 comprising providing a composition wherein the crosslinking agent has a molecular weight in a range of about 1,000-5,000.

315. The method of claim 300 comprising providing a composition wherein the activated leaving group is an N-hydroxy imide.

316. The method of claim 315 comprising providing a composition wherein the activated leaving group is N-hydroxy succinimide.

317. The method of claim 300 further comprising mixing a first mixture and a second mixture to form the composition and applying said composition to the tissue,

wherein the first mixture includes about 20-60 wt/vol% of the protein in about 0.01-0.25 molar buffer at a pH in a range of about 8.0-11.0 and the second mixture includes about 50-800 mg/ml of the crosslinking agent having a molecular weight in a range of about 1,000-15,000.

*Ans 18  
Ans Q23*

formula

318. The method of claim 317 wherein the crosslinking agent is of the formula  
G-LM-PEG-LM-G  
wherein:

-PEG- is a diradical fragment represented by the formula

$-O-(CH_2-CH_2-O)_a-$

where a is an integer from 20-300;

-LM- is a diradical fragment selected from the group consisting of a carbonate diradical of the formula,  $-C(O)-$ , a monoester diradical of the formula,  $-(CH_2)_bC(O)-$  where b is an integer from 1-5, a diester radical of the formula,  $-C(O)-(CH_2)_c-C(O)-$  where c is an integer from 2-10 and where the aliphatic portion of the diradical may be saturated or unsaturated, and a dicarbonate diradical of the formula  $-C(O)-O-(CH_2)_d-C(O)-$  where d is an integer from 2-10, or an oligomeric diradical represented by the formulas  $-R-C(O)-$ ,  $-R-C(O)-(CH_2)_c-C(O)-$ , or  $-R-C(O)-O-(CH_2)_d-O-$  where c is an integer from 2-10, d is an integer from 2-10, and R is a polymer or copolymer having 1-10 monomeric fragments selected from the group consisting of lactide, glycolide, trimethylene carbonate, caprolactone, and p-dioxanone; and

-G is the leaving group selected from the group consisting of succinimidyl, maleimidyl, phthalimidyl, imidazolyl, nitrophenyl, or tresyl.

319. The method of claim 318 wherein the protein in the first mixture is about 35-45 wt/vol% serum albumin.

320. The method of claim 319 wherein the buffer is 0.05-0.15 molar carbonate/bicarbonate buffer at a pH of about 9.0-10.5.

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321. The method of claim 318 wherein the second mixture is about 5-300 mg/ml of the crosslinking agent having a molecular weight in a range of about 1,000-5,000.

322. The method of claim 318 wherein the ratio of a volume of the first mixture to a volume of the second mixture is in a range of about 1:10 to about 10:1.

323. The method of claim 318 wherein -LM- is an oligomeric diradical  $-R-C(O)-(CH_2)_c-C(O)-$  where c is an integer from 2-10 and R is a polymer or copolymer having 1-10 monomeric fragments selected from the group consisting of lactide, glycolide, trimethylene carbonate, caprolactone, and p-dioxanone.

324. The method of claim 318 wherein -G is succinimidyl.

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325. The method of claim 318 wherein the second mixture includes about 300-800 mg/ml of a crosslinking agent having a molecular weight in a range of about 5,000-15,000.

326. The method of claim 318 wherein -LM- is a diester diradical of the formula -C(O)-(CH<sub>2</sub>)<sub>2</sub>-C(O)-.

327. The method of claim 318 wherein -LM- is a diester diradical of the formula -C(O)-(CH<sub>2</sub>)<sub>c</sub>-C(O)- where c is an integer from 2-10 and where the aliphatic portion of the diradical may be saturated or unsaturated.

328. The method of claim 318 wherein -LM- is an oligomeric diradical derived from polyglycolic acid.

329. The method of claim 300 comprising treating tissue to prevent or control a fluid leak.

*Chris Doss*  
330. The method of claim 329 wherein the fluid leak is a blood leak.

331. The method of claim 300 wherein the tissue includes an air leak.

332. The method of claim 331 wherein the air leak is in the pulmonary system.

333. A method of treating tissue to prevent formation of an adhesion comprising:

providing a composition to tissue, said composition including an albumin protein *at about 20-60 wt/vol%* and a crosslinking agent *at about 50-800 mg/ml*, said crosslinking agent having a polyoxyethylene chain portion and an activated leaving group which allows the crosslinking agent to react with said protein *and having a molecular weight in a range of about 1000-15,000*; and

curing said composition on the tissue to bond said composition to the tissue and to provide a substantive cured matrix.

334. The method of claim 333 wherein said composition is cured to produce the matrix in less than about 10 minutes.

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335. The method of claim 333 wherein said composition is cured to produce the matrix in less than about one minute.

336. The method of claim 333 wherein said composition is cured to produce the matrix in about ten seconds.

337. The method of claim 333 comprising providing the composition to the tissue using a syringe.

338. The method of claim 333 comprising providing the composition to the tissue using a dual syringe.

339. The method of claim 333 comprising providing the composition to the tissue using a spray apparatus.

340. The method of claim 333 wherein the matrix is resorbed.

341. The method of claim 340 wherein the matrix is resorbed in about four to sixty days.

342. The method of claim 333 comprising curing the composition such that the peel strength of the matrix is about 0.08 lb/in or more.

343. The method of claim 333 comprising curing said composition to provide a cured matrix that has a burst pressure greater than about 10 mmHg.

344. The method of claim 343 wherein the matrix has a burst pressure of about 34 mmHg or greater.

345. The method of claim 344 wherein the matrix has a burst pressure of about 90 mmHg or greater.

346. The method of claim 345 wherein the matrix has a burst pressure of about 130 mmHg or greater.

347. The method of claim 333 comprising providing a composition wherein the crosslinking agent has a molecular weight in a range of about 1,000-5,000.

348. The method of claim 333 comprising providing a composition wherein the activated leaving group is an N-hydroxy imide.

349. The method of claim 348 comprising providing a composition wherein the activated leaving group is N-hydroxy succinimide.

350. The method of claim 333 further comprising mixing a first mixture and a second mixture to form the composition and applying said composition to the tissue,

wherein the first mixture includes about 20-60 wt/vol% of the protein in about 0.01-0.25 molar buffer at a pH in a range of about 8.0-11.0 and the second mixture includes about 50-800 mg/ml of the crosslinking agent having a molecular weight in a range of about 1,000-15,000.

351. The method of claim 350 wherein the crosslinking agent is of the formula

G-LM-PEG-LM-G

wherein:

-PEG- is a diradical fragment represented by the formula

-O-(CH<sub>2</sub>-CH<sub>2</sub>-O)<sub>a</sub>-

where a is an integer from 20-300;

-LM- is a diradical fragment selected from the group consisting of a carbonate diradical of the formula, -C(O)-, a monoester diradical of the formula, -(CH<sub>2</sub>)<sub>b</sub>C(O)-

where b is an integer from 1-5, a diester radical of the formula, -C(O)-(CH<sub>2</sub>)<sub>c</sub>-C(O)-

where c is an integer from 2-10 and where the aliphatic portion of the diradical may be saturated or unsaturated, and a dicarbonate diradical of the formula -C(O)-O-(CH<sub>2</sub>)<sub>d</sub>-O-

C(O)- where d is an integer from 2-10, or an oligomeric diradical represented by the

formulas -R-C(O)-, -R-C(O)-(CH<sub>2</sub>)<sub>c</sub>-C(O)-, or -R-C(O)-O-(CH<sub>2</sub>)<sub>d</sub>-O- where c is an

integer from 2-10, d is an integer from 2-10, and R is a polymer or copolymer having 1-

10 monomeric fragments selected from the group consisting of lactide, glycolide,

trimethylene carbonate, caprolactone, and p-dioxanone; and

-G is the leaving group selected from the group consisting of succinimidyl, maleimidyl, phthalimidyl, imidazolyl, nitrophenyl, or tresyl.

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352. The method of claim 351 wherein the protein in the first mixture is about 35-45 wt/vol% serum albumin.

353. The method of claim 352 wherein the buffer is 0.05-0.15 molar carbonate/bicarbonate buffer at a pH of about 9.0-10.5.

354. The method of claim 351 wherein the second mixture is about 5-300 mg/ml of the crosslinking agent having a molecular weight in a range of about 1,000-5,000.

355. The method of claim 351 wherein the ratio of a volume of the first mixture to a volume of the second mixture is in a range of about 1:10 to about 10:1.

356. The method of claim 351 wherein -LM- is an oligomeric diradical -R-C(O)-(CH<sub>2</sub>)<sub>c</sub>-C(O)- where c is an integer from 2-10 and R is a polymer or copolymer having 1-10 monomeric fragments selected from the group consisting of lactide, glycolide, trimethylene carbonate, caprolactone, and p-dioxanone.

357. The method of claim 351 wherein -G is succinimidyl.

358. The method of claim 351 wherein the second mixture includes about 300-800 mg/ml of a crosslinking agent having a molecular weight in a range of about 5,000-15,000.

359. The method of claim 351 wherein -LM- is a diester diradical of the formula -C(O)-(CH<sub>2</sub>)<sub>2</sub>-C(O)-.

360. The method of claim 351 wherein -LM- is a diester diradical of the formula -C(O)-(CH<sub>2</sub>)<sub>c</sub>-C(O)- where c is an integer from 2-10 and where the aliphatic portion of the diradical may be saturated or unsaturated.

361. The method of claim 351 wherein -LM- is an oligomeric diradical derived from polyglycolic acid.

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362. The method of claim 333 wherein the composition is provided to tissue at a surgical site.

363. The method of claim 333 wherein the composition is provided on a surface of an internal organ.

364. A method of treating tissue to bind layers of tissue together comprising:

providing a composition to tissue, said composition including an albumin protein of about 20-60 wt/vol% and a crosslinking agent at about 50-800 mg/ml, said crosslinking agent having a polyoxyethylene chain portion and an activated leaving group which allows the crosslinking agent to react with said protein having a molecular weight in a range of about 1000-15,000; and

curing said composition on the tissue to bond said composition to the tissue and to provide a substantive cured matrix.

365. The method of claim 364 wherein said composition is cured to produce the matrix in less than about 10 minutes.

366. The method of claim 364 wherein said composition is cured to produce the matrix in less than about one minute.

367. The method of claim 364 wherein said composition is cured to produce the matrix in about ten seconds.

368. The method of claim 364 comprising providing the composition to the tissue using a syringe.

369. The method of claim 364 comprising providing the composition to the tissue using a dual syringe.

370. The method of claim 364 comprising providing the composition to the tissue using a spray apparatus.

371. The method of claim 364 wherein the matrix is resorbed.

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372. The method of claim 371 wherein the matrix is resorbed in about four to sixty days.

373. The method of claim 364 comprising curing the composition such that the peel strength of the matrix is about 0.08 lb/in or more.

374. The method of claim 364 comprising curing said composition to provide a cured matrix that has a burst pressure greater than about 10 mmHg.

375. The method of claim 374 wherein the matrix has a burst pressure of about 34 mmHg or greater.

376. The method of claim 375 wherein the matrix has a burst pressure of about 90 mmHg or greater.

377. The method of claim 376 wherein the matrix has a burst pressure of about 130 mmHg or greater.

378. The method of claim 364 comprising providing a composition wherein the crosslinking agent has a molecular weight in a range of about 1,000-5,000.

379. The method of claim 364 comprising providing a composition wherein the activated leaving group is an N-hydroxy imide.

380. The method of claim 379 comprising providing a composition wherein the activated leaving group is N-hydroxy succinimide.

381. The method of claim 364 further comprising mixing a first mixture and a second mixture to form the composition and applying said composition to the tissue,

wherein the first mixture includes about 20-60 wt/vol% of the protein in about 0.01-0.25 molar buffer at a pH in a range of about 8.0-11.0 and the second mixture includes about 50-800 mg/ml of the crosslinking agent having a molecular weight in a range of about 1,000-15,000.

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382. The method of claim 381 wherein the crosslinking agent is of the formula

G-LM-PEG-LM-G

wherein:

-PEG- is a diradical fragment represented by the formula

-O-(CH<sub>2</sub>-CH<sub>2</sub>-O-)<sub>a</sub>-

where a is an integer from 20-300;

-LM- is a diradical fragment selected from the group consisting of a carbonate diradical of the formula, -C(O)-, a monoester diradical of the formula, -(CH<sub>2</sub>)<sub>b</sub>C(O)- where b is an integer from 1-5, a diester radical of the formula, -C(O)-(CH<sub>2</sub>)<sub>c</sub>-C(O)- where c is an integer from 2-10 and where the aliphatic portion of the diradical may be saturated or unsaturated, and a dicarbonate diradical of the formula -C(O)-O-(CH<sub>2</sub>)<sub>d</sub>-O-C(O)- where d is an integer from 2-10, or an oligomeric diradical represented by the formulas -R-C(O)-, -R-C(O)-(CH<sub>2</sub>)<sub>c</sub>-C(O)-, or -R-C(O)-O-(CH<sub>2</sub>)<sub>d</sub>-O- where c is an integer from 2-10, d is an integer from 2-10, and R is a polymer or copolymer having 1-10 monomeric fragments selected from the group consisting of lactide, glycolide, trimethylene carbonate, caprolactone, and p-dioxanone; and

-G is the leaving group selected from the group consisting of succinimidyl, maleimidyl, phthalimidyl, imidazolyl, nitrophenyl, or tresyl.

383. The method of claim 382 wherein the protein in the first mixture is about 35-45 wt/vol% serum albumin.

384. The method of claim 383 wherein the buffer is 0.05-0.15 molar carbonate/bicarbonate buffer at a pH of about 9.0-10.5.

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385. The method of claim 382 wherein the second mixture is about 5-300 mg/ml of the crosslinking agent having a molecular weight in a range of about 1,000-5,000.

386. The method of claim 382 wherein the ratio of a volume of the first mixture to a volume of the second mixture is in a range of about 1:10 to about 10:1.

387. The method of claim 382 wherein -LM- is an oligomeric diradical -R-C(O)-(CH<sub>2</sub>)<sub>c</sub>-C(O)- where c is an integer from 2-10 and R is a polymer or copolymer

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having 1-10 monomeric fragments selected from the group consisting of lactide, glycolide, trimethylene carbonate, caprolactone, and p-dioxanone.

388. The method of claim 382 wherein -G is succinimidyl.

389. The method of claim 382 wherein the second mixture includes about 300-800 mg/ml of a crosslinking agent having a molecular weight in a range of about 5,000-15,000.

390. The method of claim 382 wherein -LM- is a diester diradical of the formula -C(O)-(CH<sub>2</sub>)<sub>2</sub>-C(O)-.

391. The method of claim 382 wherein -LM- is a diester diradical of the formula -C(O)-(CH<sub>2</sub>)<sub>c</sub>-C(O)- where c is an integer from 2-10 and where the aliphatic portion of the diradical may be saturated or unsaturated.

392. The method of claim 382 wherein -LM- is an oligomeric diradical derived from polyglycolic acid.

393. The method of claim 364 wherein the matrix binds tissue together in addition to a suture, a staple, a tape, or a bandage.

394. The method of claim 364 wherein the composition is provided to attach skin grafts.

395. The method of claim 364 wherein the composition is provided to attach adjacent layers of tissue.

396. The method of claim 364 wherein the composition is provided to position tissue flaps.

397. The method of claim 364 wherein the composition is provided to close gingival flaps.

398. A method of treating tissue comprising:

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providing a composition to tissue, said composition including an albumin protein and a crosslinking agent at about 20-60 wt/vol%, said crosslinking agent of about 50-800 mg/ml having a polyoxyethylene chain portion and an activated leaving group which allows the crosslinking agent to react with said protein and having a molecular weight in a range of about 1000-15,000; and

curing said composition on the tissue to bond said composition to the tissue and to provide a substantive cured matrix.

399. The method of claim 398 wherein said composition is cured to produce the matrix in less than about 10 minutes.

400. The method of claim 398 wherein said composition is cured to produce the matrix in less than about one minute.

401. The method of claim 398 wherein said composition is cured to produce the matrix in about ten seconds.

402. The method of claim 398 comprising providing the composition to the tissue using a syringe.

403. The method of claim 398 comprising providing the composition to the tissue using a dual syringe.

404. The method of claim 398 comprising providing the composition to the tissue using a spray apparatus.

405. The method of claim 398 wherein the matrix is resorbed.

406. The method of claim 405 wherein the matrix is resorbed in about four to sixty days.

407. The method of claim 398 comprising curing the composition such that the peel strength of the matrix is about 0.08 lb/in or more.

408. The method of claim 398 comprising curing said composition to provide a cured matrix that has a burst pressure greater than about 10 mmHg.

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409. The method of claim 374 wherein the matrix has a burst pressure of about 34 mmHg or greater.

410. The method of claim 375 wherein the matrix has a burst pressure of about 90 mmHg or greater.

411. The method of claim 376 wherein the matrix has a burst pressure of about 130 mmHg or greater.

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412. The method of claim 398 comprising providing a composition wherein the crosslinking agent has a molecular weight in a range of about 1,000-5,000.

413. The method of claim 398 comprising providing a composition wherein the activated leaving group is an N-hydroxy imide.

414. The method of claim 413 comprising providing a composition wherein the activated leaving group is N-hydroxy succinimide.

415. The method of claim 398 further comprising mixing a first mixture and a second mixture to form the composition and applying said composition to the tissue,

wherein the first mixture includes about 20-60 wt/vol% of the protein in about 0.01-0.25 molar buffer at a pH in a range of about 8.0-11.0 and the second mixture includes about 50-800 mg/ml of the crosslinking agent having a molecular weight in a range of about 1,000-15,000.

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*Chs D31*

formula

416. The method of claim 415 wherein the crosslinking agent is of the formula G-LM-PEG-LM-G wherein:

-PEG- is a diradical fragment represented by the formula -O-(CH<sub>2</sub>-CH<sub>2</sub>-O-)<sub>a</sub>- where a is an integer from 20-300;

-LM- is a diradical fragment selected from the group consisting of a carbonate diradical of the formula, -C(O)-, a monoester diradical of the formula, -(CH<sub>2</sub>)<sub>b</sub>C(O)-

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where b is an integer from 1-5, a diester radical of the formula, -C(O)-(CH<sub>2</sub>)<sub>c</sub>-C(O)- where c is an integer from 2-10 and where the aliphatic portion of the diradical may be saturated or unsaturated, and a dicarbonate diradical of the formula -C(O)-O-(CH<sub>2</sub>)<sub>d</sub>-O-C(O)- where d is an integer from 2-10, or an oligomeric diradical represented by the formulas -R-C(O)-, -R-C(O)-(CH<sub>2</sub>)<sub>c</sub>-C(O)-, or -R-C(O)-O-(CH<sub>2</sub>)<sub>d</sub>-O- where c is an integer from 2-10, d is an integer from 2-10, and R is a polymer or copolymer having 1-10 monomeric fragments selected from the group consisting of lactide, glycolide, trimethylene carbonate, caprolactone, and p-dioxanone; and

-G is the leaving group selected from the group consisting of succinimidyl, maleimidyl, phthalimidyl, imidazolyl, nitrophenyl, or tresyl.

**417.** The method of claim 416 wherein the protein in the first mixture is about 35-45 wt/vol% serum albumin.

**418.** The method of claim 417 wherein the buffer is 0.05-0.15 molar carbonate/bicarbonate buffer at a pH of about 9.0-10.5.

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**419.** The method of claim 416 wherein the second mixture is about 5-300 mg/ml of the crosslinking agent having a molecular weight in a range of about 1,000-5,000.

**420.** The method of claim 416 wherein the ratio of a volume of the first mixture to a volume of the second mixture is in a range of about 1:10 to about 10:1.

**421.** The method of claim 416 wherein -LM- is an oligomeric diradical -R-C(O)-(CH<sub>2</sub>)<sub>c</sub>-C(O)- where c is an integer from 2-10 and R is a polymer or copolymer having 1-10 monomeric fragments selected from the group consisting of lactide, glycolide, trimethylene carbonate, caprolactone, and p-dioxanone.

**422.** The method of claim 416 wherein -G is succinimidyl.

**423.** The method of claim 416 wherein the second mixture includes about 300-800 mg/ml of a crosslinking agent having a molecular weight in a range of about 5,000-15,000.

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424. The method of claim 416 wherein -LM- is a diester diradical of the formula -C(O)-(CH<sub>2</sub>)<sub>2</sub>-C(O)-.

425. The method of claim 416 wherein -LM- is a diester diradical of the formula -C(O)-(CH<sub>2</sub>)<sub>c</sub>-C(O)- where c is an integer from 2-10 and where the aliphatic portion of the diradical may be saturated or unsaturated.

426. The method of claim 416 wherein -LM- is an oligomeric diradical derived from polyglycolic acid.

427. The method of claim 398 comprising curing the composition on the tissue to seal the tissue.

428. The method of claim 427 comprising treating tissue to prevent or control a fluid leak.

429. The method of claim 428 wherein the fluid leak is a blood leak.

430. The method of claim 427 wherein the tissue includes an air leak.

431. The method of claim 430 wherein the air leak is in the pulmonary system.

432. The method of claim 398 wherein the composition is provided to tissue at a surgical site.

433. The method of claims 398 comprising curing the composition at the tissue to prevent a tissue adhesion.

434. The method of claim 398 wherein the composition is provided on a surface of an internal organ.

435. The method of claim 398 comprising curing the composition to form a matrix to bind tissue.

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*Conclude*

436. The method of claim 435 wherein the matrix binds tissue together in addition to a suture, a staple, a tape, or a bandage.

437. The method of claim 398 wherein the composition is provided to attach skin grafts.

438. The method of claim 398 wherein the composition is provided to attach adjacent layers of tissue.

439. The method of claim 398 wherein the composition is provided to position tissue flaps.

440. The method of claim 398 wherein the composition is provided to close gingival flaps.